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Friend Turned Foe: Evolution of Enterococcal Virulence and Antibiotic Resistance

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Abstract

The enterococci are an ancient genus that evolved along with the tree of life. These intrinsically rugged bacteria are highly adapted members of the intestinal consortia of a range of hosts that spans the animal kingdom. Enterococci are also leading opportunistic hospital pathogens, causing infections that are often resistant to treatment with most antibiotics. Despite the importance of enterococci as hospital pathogens, the vast majority live outside of humans, and nearly all of their evolutionary history took place before the appearance of modern humans. Because hospital infections represent evolutionary end points, traits that exacerbate human infection are unlikely to have evolved for that purpose. However, clusters of traits have converged in specific lineages that are well adapted to colonize the antibiotic-perturbed gastrointestinal tracts of patients and that thrive in the hospital environment. Here we discuss these traits in an evolutionary context, as well as how comparative genomics is providing new insights into the evolution of the enterococci.

Keywords

Enterococcus; genomics; human-associated microbial flora

Introduction: Origins and Evolution of the Enterococci

Enterococci predate the split of humans from primates by aeons, but these bacteria were not specifically noticed until the end of the nineteenth century, when Thiercelin (117) and MacCallum & Hastings (66) separately described human enterococcal infections. After more than a hundred years, the experimental proof of Koch's postulates by the latter still stands as one of the finest studies in the field (66). Nearly all research on enterococci has been in the context of human infection, with comparatively little attention paid to their wide distribution or roles in nature. Yet it is only through the lens of their native biology—the result of evolution over millennia—that we are likely to understand their emergence as hospital

pathogens (39, 129) capable of transmitting antibiotic resistances to other clinically important microbes (55, 64).

The genus *Enterococcus* consists of nonsporulating gram-positive bacteria that were initially grouped within the genus *Streptococcus* (65). The genus was officially named *Enterococcus* in 1984, based on sequence divergence of 16S rRNA (102). Enterococci inhabit the intestinal tracts of humans, animals, and insects (8, 20, 71, 72, 77) and the environments these organisms inhabit, including plants, soil, and water (40, 78). The most parsimonious explanation for their occurrence in this vast array of hosts is that they were core members of the intestinal microbiome of the last common ancestor of mammals, reptiles, birds, and insects, which existed in the early Devonian period, over 400 mya (39) (Figure 1).

Given the broad distribution of enterococci among animals and in the environment, the fraction of enterococci that reside within humans is very small. Furthermore, as a core member and the most abundant gram-positive coccus in the human gastrointestinal (GI) tract (58, 124), the fraction of human-associated enterococci causing infection is infinitesimally small. It is therefore unlikely that any particular trait found in enterococci outside of hospitals evolved or was acquired because of its ability to exacerbate disease. Rather, native enterococcal factors that contribute to disease exist because of their contribution to bacterial survival and proliferation in one or more natural habitats. Ironically, we currently know almost nothing about these natural roles.

Enterococci are ubiquitous members of GI tract consortia. This may be because of their ability to survive harsh innate defense systems of host GI tract environments, including the grinder of one of the oldest and most abundant forms of animal life, nematodes (36). The ability to colonize the intestine of nematodes, where they are bathed in the lysates of other bacteria, would provide enterococci with a nutrient-rich habitat. This may have contributed to initial host adaptation, resulting in the streamlined genomes observed today. Enterococci are auxotrophic for many amino acids, vitamins, and micronutrients (84), and the genomes of commensal strains are only about 2.75 megabases (88). Enterococci are also able to survive and grow in a wide range of temperatures and pHs and in both hypertonic and hypotonic conditions (106, 107).

As a typical omnivorous mammal, humans shed the equivalent of their entire intestinal microbiota into the environment every 2–3 weeks (37, 130). With an average historic life span of 25–30 years (52), this corresponds to depositing more than 500 colon equivalents into the environment over a lifetime. Therefore, far less than 1% of human-derived enterococci actually reside within the human GI tract at any given time (or, looked at another way, a small fraction of the enterococcal life cycle is spent in the human, let alone causing disease). Enterococci are able to survive in sediments and soils, on the surface of aquatic and terrestrial vegetation, and in freshwater and marine water (10, 111), and they persist in these settings longer than most bacteria (110). Despite the auxotrophies of enterococci, some studies indicate that they are able to replicate in the environment (27, 134), possibly as the result of collaboration within a polymicrobial consortium. This survival ability results from their tolerance of UV irradiation (70), salt, starvation (43), and possibly

predation by protozoa (44) and bacteriophages (93). This inherent ruggedness has no doubt contributed to their emergence in the harsh environment of the modern hospital.

Nearly 50 species of enterococci have been identified, mostly in the genomic era (90) (Figure 2). Speciation occurs when populations become isolated and diverge over time (73). We currently know little about the natural distribution of enterococcal species or the ecological isolation that led to speciation. *E. faecalis* and *E. faecium* are the most common species isolated from the human GI tract, with the former being predominant (69, 85). They are also the enterococci most often associated with human infection (39). It is therefore surprising that these two species are at opposite ends of the phylogenetic tree (Figure 2). *E. faecalis* occurs in one of the oldest branches of the genus, whereas *E. faecium* arose more recently. However, their common occurrence in the human intestine and in hospital infection shows that they possess common features that allow them to inhabit similar ecologies. Given that both species share overlapping, if not identical, microecologies within humans, it is clear that the isolation required for speciation of *E. faecium* did not occur there.

What drives enterococcal speciation? The specific nature of an animal's intestinal tract (microbial diversity, diet, host defense and other secretions, body temperature, pH) selects for the outgrowth of favorably matched strains, resulting in host adaptation (22, 69). In intestinal tracts that are not ecologically isolated, such as the intestinal tracts of an insect and insectivore (where enterococci of predator and prey coningle in the GI tract of the predator), separate species may not evolve, because of horizontal movement of genes. However, enterococci within the intestinal tract of an herbivore may be sufficiently isolated from other members of the genus to permit speciation—the ultimate extension of host adaptation.

The lack of clade structure, or even substantial genetic drift among populations of *E. faecalis* (54, 88), might be explained by the observation that it is widely disseminated along the food chain (71, 77). It is tempting to speculate that this stems from genetic mixing between enterococci of predator and prey, possibly facilitated by the near-exclusive occurrence of highly efficient pheromone-responsive plasmid gene transfer systems in *E. faecalis* (30, 31). It was recently shown that large pieces of the chromosome could in fact be mobilized and transferred between *E. faecalis* strains by pheromone-responsive plasmids (68), possibly limiting divergence and speciation.

The relative ecological isolation of the gut flora of herbivores leads to two predictions: First, a main driver of *Enterococcus* speciation may have been the large radiation in mammalian herbivore species that accompanied the global expansion of C4 photosynthesizing plants eight to three million years ago (32). Second, more species divergence is likely to occur among the host-adapted enterococci of various herbivores. The occurrence in the phylogenetic tree of *E. faecium* as a modern species, along with considerable branching and divergence, suggests its genesis at a time of considerable host species radiation (Figure 2).

Evolution of Enterococcal Virulence Factors

Virulence is traditionally defined as the extent to which an organism can cause disease (11). It is often viewed as a means to an end—enhanced growth and dissemination to other hosts (57, 118). However, enterococcal infection in humans and close relatives with a long history

of hygienic funerary rituals (95) is an evolutionary dead end. In other words, because there is little chance of bloodstream-to-bloodstream or heart-valve-to-heart-valve transmission of enterococci, there is no sustained advantage for them to proliferate at these common sites of infection (9). Although enterococci can cause infection in nonhospitalized patients, outside of the hospital setting this is rare and often results from an underlying predisposition. This appears to have been the case for the first *Enterococcus*-infected patient, described by MacCallum & Hastings (66). The patient had a prior history of rheumatic fever, which may have caused heart valve scarring and blood flow abnormalities, predisposing him to endocarditis. Almost 10 years later, he developed and succumbed to enterococcal endocarditis, perhaps related to a recent severe respiratory infection (66). Although in this classic case the patient was not infected in a hospital, most invasive enterococcal infections today occur within hospitals, in patients with underlying conditions and whose native flora has been destabilized by prior antibiotic use (39).

Not all enterococci are equal. The bulk of the multidrug-resistant enterococcal infection problem is caused by discrete lineages of *E. faecalis* and *E. faecium*, and we now know the principal characteristics of those lineages (54, 60, 74, 79, 100, 112). In the case of *E. faecalis*, hospital-adapted, multidrug-resistant lineages include strains of multiple-locus sequence type clonal clusters CC2, CC9, CC28, and CC40 (74, 100, 112). For *E. faecium*, most hospital-acquired infections are caused by CC17 and closely related strains (54, 60). What, then, makes these lineages different from commensal *E. faecalis* and *E. faecium* strains? In both species, a variety of auxiliary traits have converged on mobile elements, resulting in genomes that are significantly larger than those of commensal strains (61, 88). The hospital-adapted clonal lineages are also unique in their ability to be transmitted patient-to-patient within the hospital setting, being well suited to thrive in that environment (2).

Because invasive infection is an evolutionary end point, the hospital environment has selected for traits that make colonization and transmission more likely. As a result, the variable traits that have converged in hospital-adapted, multidrug-resistant enterococci include antibiotic resistances (61, 74, 112), an Esp-containing pathogenicity island (59, 61, 104, 112), and more complex integral cell wall carbohydrate operons (88, 112). In the prototype CC2 *E. faecalis* strain, V583, a protein termed aggregation substance (AS) is encoded by antibiotic-resistance plasmids, and additional alleles occur on the chromosome (91). AS is a prototypical example of an enterococcal virulence factor, in that it exacerbates various measures of infection (15, 33, 114). But because invasive enterococcal infection is an evolutionary end point, it is more likely that the presence of AS in hospital lineages of *E. faecalis* stems from its contribution to GI tract colonization, mainly through association with antibiotic-resistance plasmids [but also possibly because of its ability to increase binding to colonic epithelial cells (56, 86)]. AS is an LPXTG-containing surface protein that is an important component of pheromone-responsive plasmid transfer systems of *E. faecalis* (30, 31). Briefly, peptide pheromones are secreted by potential *E. faecalis* plasmid recipient cells, and these pheromones are sensed by *E. faecalis* cells harboring pheromone-responsive plasmids. Induction of AS expression promotes effective mating pair formation, a first step in plasmid transfer. During infection, AS increases vegetation size and severity of *E. faecalis* experimental endocarditis (14, 16) and promotes internalization of bacteria by

cultured enterocytes (127). AS also contributes to biofilm formation, which results in bacterial adherence and persistence on tissues and medical devices (16). Its association with postsegregational killing systems on pheromone-responsive plasmids, which also often contain antibiotic-resistance genes, likely selects for the maintenance of AS within cells. These postsegregational killing systems promote retention of antibiotic-resistance plasmids, even in the absence of antibiotic selection (17, 41).

Another virulence trait that undoubtedly evolved for other purposes is the cytolysin of *E. faecalis*. The cytolysin is a broadly active bacteriocin that is effective against nearly all species of gram-positive bacteria (123). Originally described in the 1930s (119), the cytolysin is now known to belong to the large and diverse class of lantibiotic peptides (132). Yet even among lantibiotics, the cytolysin is unique in its stereochemistry (115), as well as in its ability to lyse erythrocytes and other eukaryotic cells, in addition to bacteria (19). The cytolysin contributes to enterococcal virulence in a wide array of infection models (14, 36, 46, 50) and is found on the pathogenicity island initially characterized in the *E. faecalis* CC2 strain MMH594 (104), or on pheromone-responsive, AS-expressing plasmids, such as pAD1 (45). Like AS, the cytolysin is found in both clinical and other *E. faecalis* strains (7, 47). The cytolysin probably evolved initially as a colonization factor, potentially in a nonhuman host, and likely allowed *E. faecalis* to access new ecological space via its bacteriocin activity (99). It is currently unknown whether the ability to lyse eukaryotic cells is an evolved function or simply a consequence of the broad antiprokaryotic activity of this molecule. Speculatively, the ability to lyse eukaryotic cells may have allowed for the killing of eukaryotic intestinal parasites in an insect or other primitive host, which would confer a selective advantage to bacterium and host, a formula for evolutionary success.

A third factor that has converged in multidrug-resistant, hospital-adapted enterococcal lineages of both *E. faecalis* and *E. faecium*, but that likely evolved in response to other selective forces, is the surface protein Esp (105). As noted above, Esp is encoded within collections of genes that have converged in pathogenic lineages on pathogenicity islands (105); and importantly, Esp occurs in the leading hospital lineages of both *E. faecalis* and *E. faecium* (105, 131). A version of Esp has also been identified in some strains of *Staphylococcus aureus* (75). In *S. aureus* and in enterococci, Esp promotes tissue attachment and biofilm formation (120, 121). This attachment contributes to persistence and survival at sites of infection and also likely plays a similar role at sites of commensal colonization in other hosts, although this has not been studied extensively.

The structure of the cell wall also appears to be enhanced in hospital-adapted lineages: *E. faecalis* strains belonging to CC2, CC9, and CC28 (but not CC40) express different versions of the capsular polysaccharide (42, 74, 112). This trait enhances *E. faecalis* resistance to phagocytosis (42) and has not been identified in *E. faecium*. Because bloodstream-to-bloodstream transmission of enterococci is unlikely, this trait most likely evolved to enhance general environmental resistance, or possibly resistance to predation by amoebas, bacteriophages, or other predators. Hospital strains of both *E. faecalis* and *E. faecium* do have more elaborate operons for production of the integral cell wall carbohydrate Epa (88, 112), which may confer additional environmental resistance.

The variable features that have converged in hospital-adapted lineages occur in the context of general features of enterococci that also make them more likely to cause infection than many other species. The many background traits that occur in most enterococcal strains and also contribute to the virulence of enterococci have been extensively examined and reviewed (49, 76, 79–81, 94, 97). Among other traits, these include the adhesin Ace (21, 82, 83, 109) and the extracellular metalloprotease gelatinase, both of which are expressed by *E. faecalis* (6, 67, 96). A main role of gelatinase in nature appears to be remodeling the *E. faecalis* surface, which can include modulating surface levels of Ace in mature communities, in response to accumulation of quorum signals (92).

Evolution of Antibiotic Resistance

Antibiotics and antibiotic resistance have ancient roots that may stretch as far back as the origins of life (23). Yet our ability to produce, purify, and release hundreds of millions of tons of antibiotics into the human ecosystem has fundamentally changed the human–proximal microbial landscape (24, 63, 48). Much of the enterococcal antibiotic-resistance problem stems from horizontally acquired traits (89). Enterococci are particularly well positioned to both acquire and serve as a depot for antibiotic-resistance factors for two reasons. First, enterococci exist in complex microbial ecosystems, in intimate contact with a large diversity of potential sources of genetic material (37). Second, because of their high level of intrinsic antibiotic resistance (49, 79–81), enterococci occur in environments that are substantially enriched for antibiotic-resistance elements.

Whereas the ability of enterococci to readily pick up antibiotic resistances in a hospital setting seems obvious, there are few reasons to believe that this phenomenon is exclusive to modern hospitals. Given their wide distribution in nature, enterococci have had opportunities to acquire antibiotic resistances throughout their evolutionary history, and they likely have done so for hundreds of millions of years. Rotting plant matter is a main substrate on which many antibiotic-producing streptomycetes live, as well as a principal food source for many insects (103). Ingestion of antibiotic-producing/resistant streptomycetes, along with antibiotic-laden rotting plant matter, would give insect-resident enterococci access to the necessary genetic material, as well as a potential selective advantage, for acquiring resistance genes. Antibiotic resistance determinants identified in enterococci, such as aminoglycoside and vancomycin-resistance genes, bear obvious relationships to resistance operons from antibiotic-producing soil organisms (5, 26). Whereas in nature this is undoubtedly an exceedingly rare event, the release into the human-associated biosphere of millions of tons of antibiotics over the last 75 years provided opportunities for the outgrowth of potentially naturally occurring resistant strains, as well as strong selective pressure for spreading resistance determinants worldwide (24).

Isolates of *E. faecalis* that have ended up in strain collections, mainly from clinical infection sources, appear to have acquired resistance to tetracycline and chloramphenicol in the 1950s and 1960s, followed by gentamicin and erythromycin resistance in the 1970s, then ampicillin and vancomycin resistance in the 1980s and 1990s (Figure 3) (87). Interestingly, the occurrence of multiple acquired drug resistances in *E. faecalis* is inversely correlated with the presence of a functional clustered regularly interspaced short palindromic repeats

(CRISPR)/Cas locus. Moreover, in several *E. faecalis* strains the CRISPR/Cas loci contain spacers derived from plasmids known to transmit antibiotic resistances (87). This suggests that the CRISPR/Cas system helps protect the *E. faecalis* genome from intrusion of mobile genetic elements, and that a defective CRISPR/Cas system might allow for acquisition of mobile elements and the antibiotic resistances that they encode. Speculatively, antibiotic selection for loss of CRISPR/Cas may also have made hospital-adapted strains of *E. faecalis* more receptive to other mobile elements, including the pathogenicity island and phages (87). The role played by the CRISPR/Cas system in *E. faecium*, where it occurs less frequently, is less clear (61, 87).

The recent emergence of vancomycin resistance in hospital-adapted enterococci provides an apt illustration of the importance of understanding their natural ecology and evolutionary history. Details of key events are missing, and competing interests shift the focus back and forth regarding possible interpretations of the available data. As a result, how and why vancomycin resistance emerged among the enterococci remain somewhat controversial. The bottom line is unambiguous—you get what you select for. Like Newton's third law, because of the tremendous diversity of life, for every action that affects an ecology, there is an equal and opposite reaction. The advent of the antibiotic era has therefore provided a figurative opportunity for Darwin to meet Newton (38).

The facts are these: It is indisputable that the glycopeptide avoparcin was widely used as an animal growth promoter in Europe and Australia (51, 125), and not the United States, prior to and throughout the emergence of vancomycin-resistant enterococci (VRE) in hospitals. Outbreaks of VRE occurred first in England (122) and France (62), nearly three years before similar outbreaks occurred in the United States (101). In France, the first VRE were *E. faecium* (62), whereas England saw a mixture of mostly *E. faecium* but also some *E. faecalis* (122). Not surprisingly, the vancomycin-resistance determinants were found to occur on mobile elements (62). At the time, there were no rigorous studies of the emergence of VRE in hospitals, nor of the environments to which patients or health-care workers had been exposed prior to hospitalization, so cause and effect were not experimentally proven. However, a logical proof of cause and effect can be convincingly advanced, as detailed further below.

The appearance of VRE hospital strains stems from a series of reactions for which we have little information to accurately model. Furthermore, the spread of VRE involves yet other factors. For the first 30 years following the introduction of vancomycin, resistance in clinically important microbes was not reported. We currently know little of the rate-limiting step for vancomycin resistance to develop or the rates of each necessary reaction that occurred along the way. First, vancomycin resistance had to enter the enterococci in ecologies that overlap with hospitalized patients. The most common vancomycin-resistance determinants among hospital isolates are the VanA and VanB types (12). As shown in Figure 4a, the modern VanA operon has existed for at least 30,000 years, as have derivatives that show its evolution from the ultimate precursor in the *Amycolatopsis orientalis* vancomycin producer (25). Closely related operons have also been identified in the soil-dwelling-insect pathogen *Bacillus* (now *Paenibacillus*) *papillae* (98). Moreover, related vancomycin-resistance operons naturally occur in the genomes of *E. gallinarum* and *E.*

casseliflavus (12, 88). This is important because *E. casseliflavus* was identified in 43.5% of insects [using culture methods (71)], indicating that both pathogenic and commensal enterococci of insects have had reasons to maintain vancomycin-resistance elements for aeons.

What is the effect of applying orally consumed avoparcin to animals? It clearly enriches the glycopeptide-resistance determinants in the ecology (1). This enrichment may first occur by the selective outgrowth of microbes associated with insects living near antibiotic-containing agricultural animal excreta (e.g., microbes akin to *P. papillae* or *E. casseliflavus*). An increase in the abundance of vancomycin-resistance operons in the vicinity of animal-adapted enterococcal strains, especially in the presence of selection, increases the probability of movement of resistance into animal-adapted enterococci. Few studies have systematically examined insects or other nonmammalian ecologies in agricultural areas treated with avoparcin. However, within years of the initial reports of VRE infections, VRE were found to be common among the commensal flora of Europeans and their farm animals (4, 28), whereas they are rare among the commensal flora of farm animals in the United States (18, 108, 116, 128). Moreover, following the ban on the use of avoparcin in agriculture, there have been statistically significant decreases in the abundance of VRE associated with farm animals in Denmark (3) and France (53), strongly suggesting direct cause and effect between agricultural use of avoparcin and VRE occurrence.

The first outbreak of VRE infection began in November 1986 in Dulwich Hospital, London (122). Over the course of roughly a year, 55 enterococcal isolates were obtained from patients who had been treated typically with vancomycin every five to seven days, along with a cephalosporin. The VRE that emerged included both *E. faecium* and *E. faecalis*, indicating that this outbreak was not entirely clonal and that the vancomycin resistance was likely mobile. Nearly all strains of both species were reported to be resistant to both vancomycin and teicoplanin, a hallmark of the VanA phenotype (12, 122). Most vancomycin-resistant *E. faecium* in this outbreak were also ampicillin resistant (122) and exhibited other resistances indicative of well-adapted hospital strains. Two vancomycin-resistant *E. faecium* strains nearly simultaneously isolated in a French hospital (62) were also resistant to ampicillin and other drugs, suggesting that vancomycin resistance had entered the hospital-adapted enterococci. Around 1986, then, the VanA operon entered hospital-adapted lineages of enterococci in geographical areas where avoparcin had been used.

The first cases of VRE in the United States occurred beginning in February 1987 at Barnes Hospital in Saint Louis, Missouri (101). Although this was only months after the European cases, there were important differences. First, the first US VRE isolates were *E. faecalis*. They were resistant to multiple antibiotics (101) and were found to be prototypical CC2 hospital strains (74). However, these isolates displayed a different phenotype, being vancomycin resistant but teichoplanin susceptible (101). A similar vancomycin-resistant *E. faecalis* that exhibited teichoplanin susceptibility was nearly simultaneously isolated in France (133), and this phenotype was termed VanB (12). As with hospital-endemic strains of *E. faecium*, CC2 strains of *E. faecalis* are mainly associated with hospital environments

(74), which suggests that shortly after the movement of VanA into the enterococci, likely in Europe, VanB moved into *E. faecalis*, possibly in the United States or in Europe.

In the United States, VanB *E. faecalis* did not ultimately emerge as the main problem. Even now, only about 10% of *E. faecalis* infections in the United States are vancomycin resistant (39), whereas vancomycin-resistant *E. faecium*, mainly of the VanA phenotype, has become endemic. In the United States, the VanA outbreak appears to have started in New York City in 1989 (35). This outbreak looked very much like the one that began almost three years earlier in London. Because of the London outbreak, an active surveillance program was in place in New York City, and no VRE were detected throughout 1988. The first VRE strain was not detected until September 1989, and by October 1991, 361 patients at 38 hospitals in the New York City area were identified with VRE infections (Figure 4b). The vast majority of strains were *E. faecium*; of the *E. faecalis* strains, most were of the VanA phenotype, although a few VanB strains were noted. One patient had both *E. faecium* and *E. faecalis* VRE, and by Southern blot it appeared that vancomycin resistance in both species was mediated by related elements, suggesting horizontal transfer within the patient (35). All *E. faecium* strains were resistant to ampicillin and other antibiotics, emblematic of establishment of the VanA operon in the highly-hospital-adapted CC17 lineage. This lineage rapidly spread to become a leading cause of hospital-acquired VRE infection throughout the United States in the 1990s, before spreading globally in the 2000s (12).

The question, then, is why did the first cases of VRE occur in Europe but not become an endemic problem there until the 2000s, whereas VRE immediately took off in the United States? One likely reason is the level of vancomycin used in the United States. In the mid-1990s, physicians in the United States used about 12 times more vancomycin per capita in hospitals than did their colleagues in the Netherlands (125). Furthermore, even within the comparatively small geographic area of Europe, clinical practices vary by country. As a result, rates of VRE bloodstream infection in Luxembourg are on average 10 times those of surrounding countries (12), indicating that differences in clinical practices, not geographic isolation, are now the main drivers of VRE incidence.

There was great concern of further spread of vancomycin resistance to *S. aureus*, because vancomycin is an important last-line therapy for methicillin-resistant *S. aureus* (MRSA) infections. Surprisingly, it took over 15 years for vancomycin resistance to transfer from enterococci into *S. aureus* in clinical isolates (13, 55, 126). However, likely because of the fitness cost (34), each case of vancomycin-resistant *S. aureus* appears to have arisen de novo in each patient in which it was identified (55), and there are no well-documented cases of patient-to-patient spread. However, just as rare events gave rise to well-adapted VRE, many additional mutations may not be required in *S. aureus* to mitigate the current fitness costs.

Recent comparative genomics studies have shown that the CC17 lineage of *E. faecium* is a hypermutator strain that arose from a clade mainly inhabiting the GI tracts of domestic animals roughly 75 years ago—exactly the time of the introduction of antibiotics into medical practice (Figure 5) (61). Interestingly, the main differences between the clade from which CC17 branched and human commensal microbes, in addition to the previously mentioned accumulation of antibiotic-resistance elements, pathogenicity islands, and other

mobile elements, are differences in carbohydrate utilization pathways. Human commensal *E. faecium* strains appear to be well equipped to metabolize carbon derived from dietary sources. However, animal strains and the hospital-endemic CC17 lineage have traded many of these operons for pathways for metabolizing amino sugars of the type found on the surface of gut epithelial cells and in secretions such as mucin (54, 61, 135). In other words, human commensal strains appear to be exactly that—sharing at the table with the host. Animal and hospital *E. faecium* strains, however, appear to have evolved the ability to utilize host secretions and cell surface modifications as carbohydrate sources in a more parasitic relationship. The divergence between human and animal *E. faecium* strains appears to have started a little more than 3,000 years ago—a time of increasing urbanization of humans, increasing domestication of animals, and introduction of hygiene measures (61). Conceivably, forcing animals onto a restricted diet through domestication may have been an important driving force in the evolution of animal strains of *E. faecium*. Rather than deriving carbon from dietary sources that no longer existed because of dietary shifts imposed by domestication, they evolved to derive carbon from host secretions. Coincidentally, this would also be an asset in colonizing the GI tract of a hospitalized patient in the intensive care unit, where feeding may be by intravenous administration, with few sources of carbon other than host secretions in the GI tract. Experimental results are emerging that support this prospect (135).

Conclusion

Since the discovery of the enterococci most attention has been paid to the anomalous biology of a small number of lineages that are associated with human infection. Relatively few studies have aimed to contribute to an understanding of the natural history and ecology of the enterococci. Understanding the forces that have shaped the evolution of the enterococci over aeons will enable us to learn how these resilient bacteria have adapted to the unique conditions of the hospital environment in the antibiotic era.

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Glossary

Devonian period	a geologic period spanning approximately 420–360 mya
Coccus	a spherical bacterium
Hypertonic	an environment with a higher concentration of solutes outside the cell than inside the cell, which causes water to flow out of the cell by osmosis
Hypotonic	an environment with a higher concentration of solutes inside the cell than outside the cell, which causes water to flow into the cell by osmosis
Protozoa	single-celled eukaryotic organisms
Bacteriophages	viruses that infect and replicate within bacteria
Phylogenetic tree	a branching diagram that shows the evolutionary relationships between species, based on differences in their genetic characteristics
Endocarditis	infection caused by inflammation of the heart lining and heart valves
Streptomycetes	actinobacteria found predominantly in soil and decaying vegetation that produce the majority of clinically useful antibiotics of natural origin
Avoparcin	a glycopeptide antibiotic used in agriculture that shares chemical similarity with vancomycin

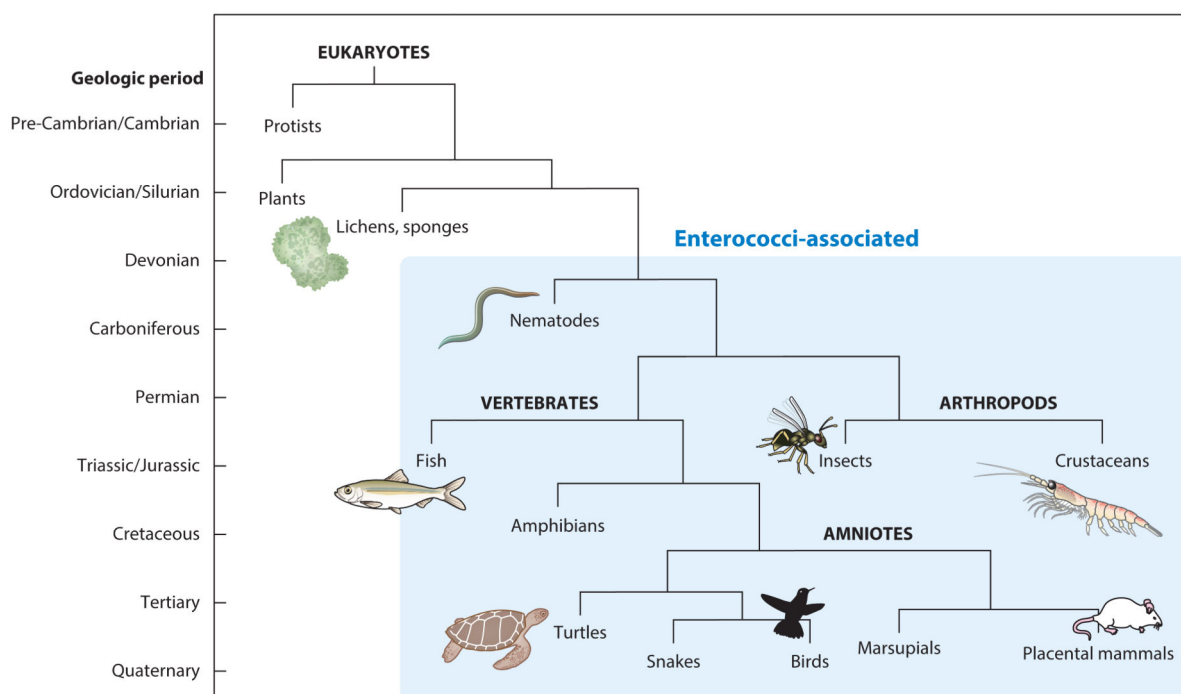


Figure 1. Simplified tree of eukaryotic life, with blue shading indicating animals with which enterococci have been found to be associated. The labels to the left of the tree show approximate geologic periods.

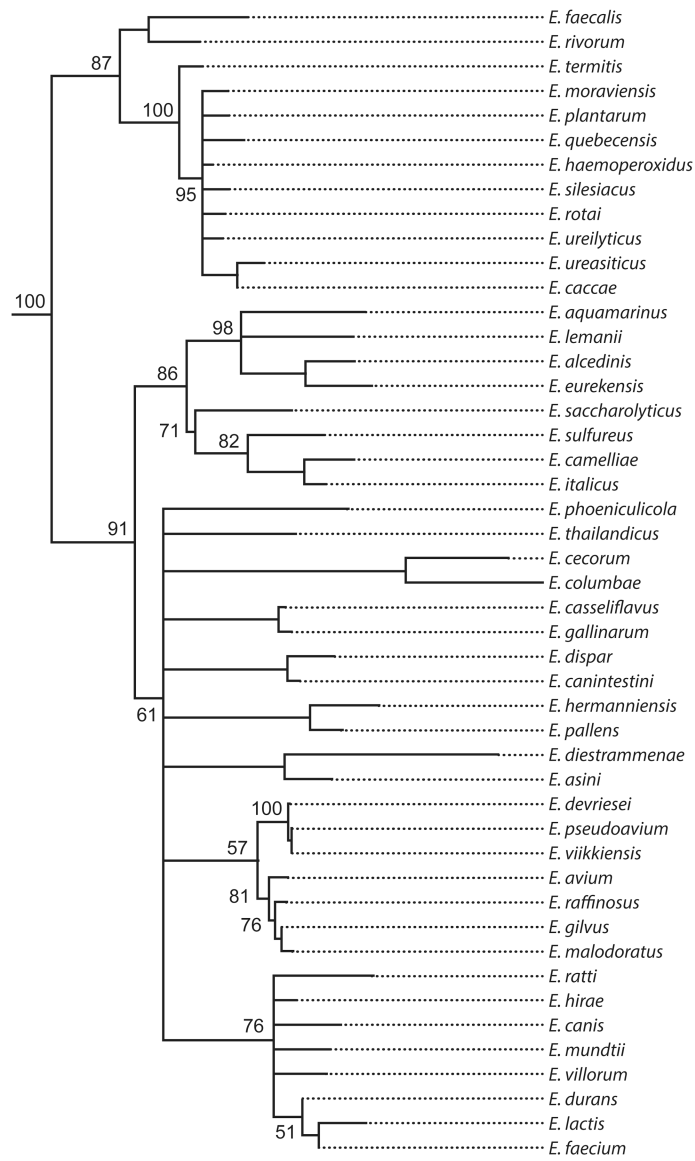


Figure 2.

Phylogenetic tree of the genus *Enterococcus*. Available 16S rRNA gene sequences for each species were compiled using Geneious software (Biomatters, Ltd., San Francisco, California) with a neighbor-joining algorithm. The 16S rRNA sequence of *Vagococcus lutrae* was used as an outgroup. Bootstrap values are shown for nodes with more than two branches and were generated over 1,000 iterations.

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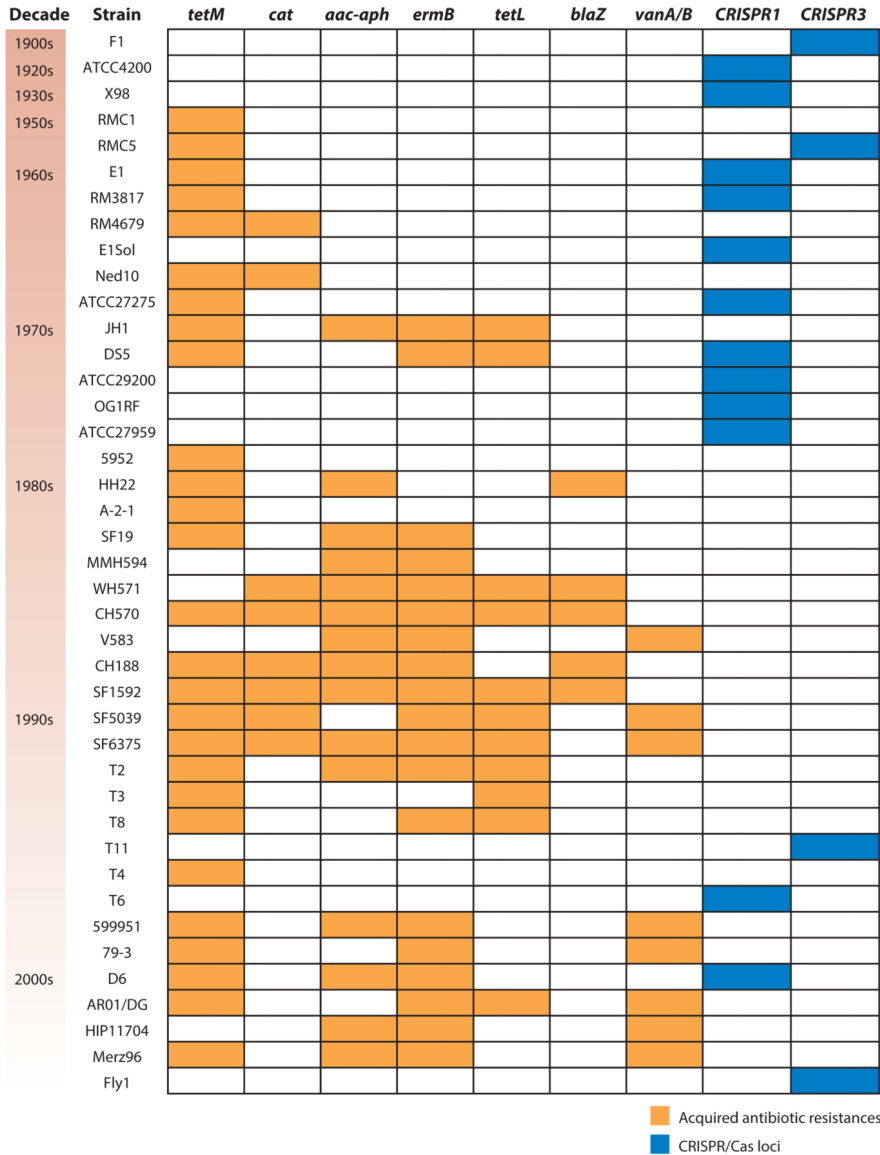
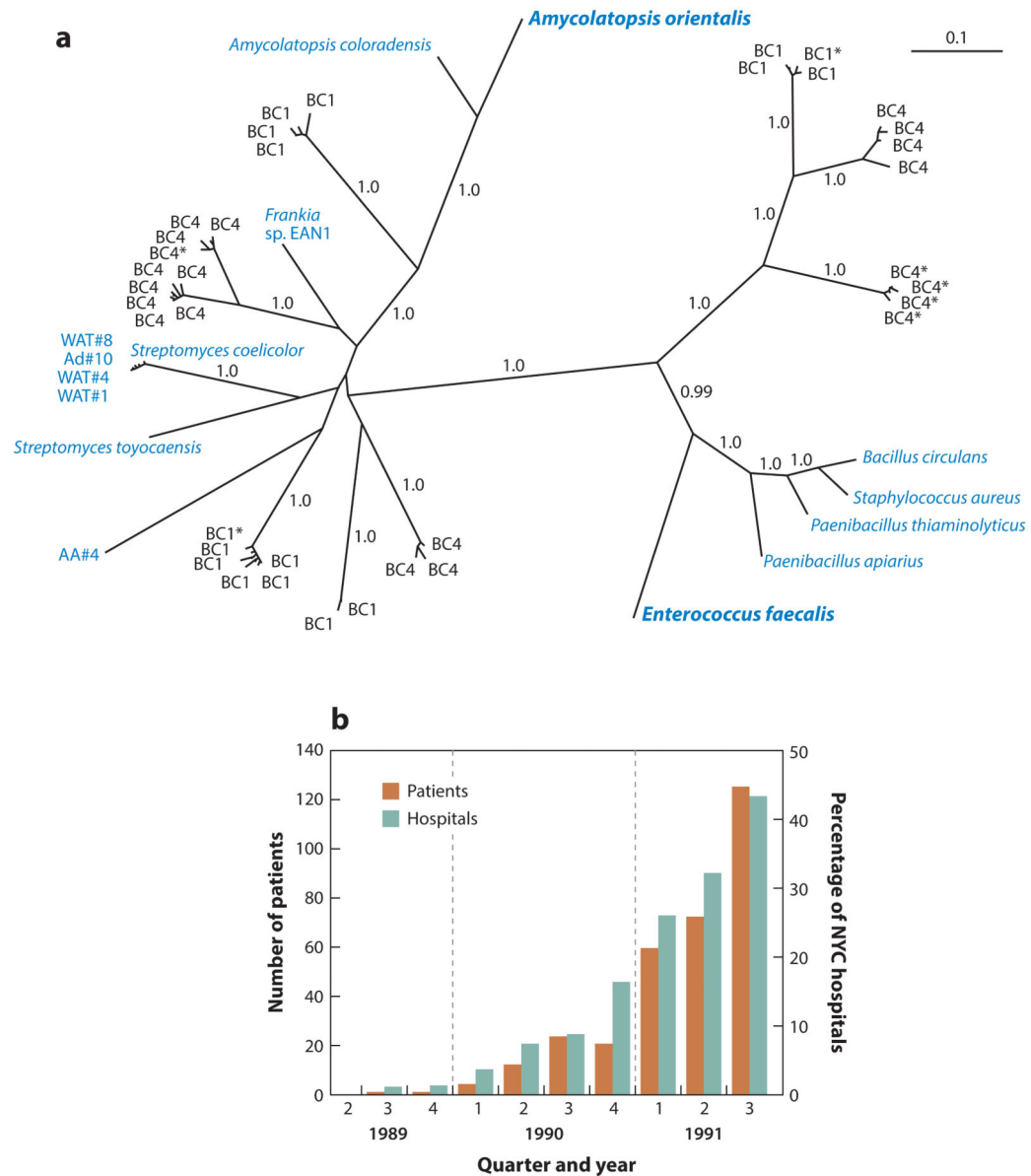


Figure 3. Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas loci and acquired antibiotic resistances in *Enterococcus faecalis*. Strains are ordered by date of isolation, from oldest to most recent, and approximate decades of isolation are shown to the left of the strain names. Presence of acquired antibiotic resistances is shown with orange shading, and CRISPR/Cas locus presence is shown in blue. Only strains containing an acquired antibiotic resistance or a CRISPR/Cas locus are included. Antibiotic resistances were profiled in Reference 74 and correspond to the following compounds: tetracycline (*tetM* and *tetL*), chloramphenicol (*cat*), gentamicin (*aac-aph*), erythromycin (*ermB*), ampicillin (*blaZ*), and vancomycin (*vanA/B*). Figure is adapted from Reference 87.

**Figure 4.**

(a) Unrooted Bayesian phylogeny of translated *vanA* sequences analyzed in Reference 25. Blue denotes strains with VanHAX clusters confirmed to confer resistance to vancomycin; sequences containing stop codons but homology throughout are noted with a single asterisk. The scale bar represents 0.1 substitutions per site. Panel is reproduced from Reference 25.

(b) Number of patients and cumulative percentage of New York City (NYC) hospitals with vancomycin-resistant enterococci infection between 1989 and 1991. Panel is reproduced from Reference 35.

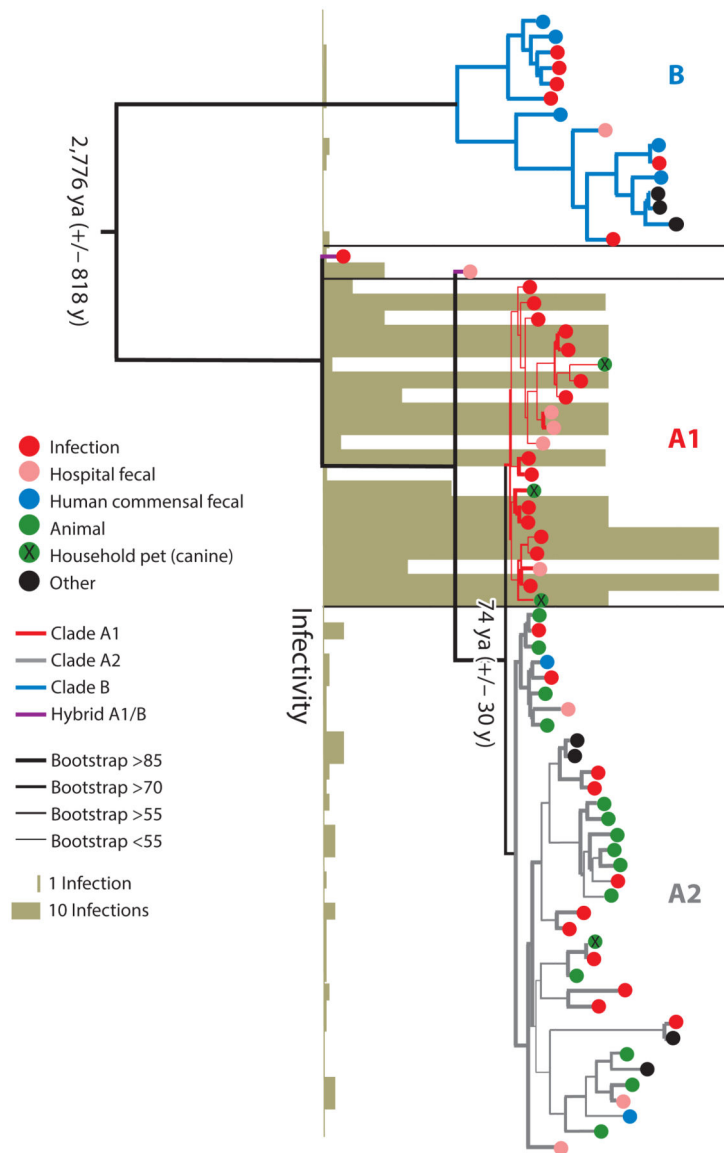


Figure 5.

RAxML (113) single-nucleotide-polymorphism-based tree based on concatenated alignments of DNA sequences of 1,344 single-copy core genes in 73 *Enterococcus faecium* genomes. Bootstrapping was performed with 1,000 replicates. The origins of the strains and the dates for the split between the clades, estimated by BEAST analysis (29), are indicated. The infectivity score reflects the number of strains of a particular sequence type, in the MLST database, isolated from infection. Three clades are highlighted: B in blue, A1 in red, and A2 in grey. Figure is reproduced from Reference 61.